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DOI:
10.1080/15412555.2018.1476475

Document Version
Peer reviewed version

Citation for published version (Harvard):
Butler, A, Walton, GM & Sapey, E 2018, 'Neutrophilic inflammation in the pathogenesis of Chronic Obstructive Pulmonary Disease', *COPD: Journal of Chronic Obstructive Pulmonary Disease*, vol. 15, no. 4, pp. 392-404. https://doi.org/10.1080/15412555.2018.1476475

Neutrophilic Inflammation in the Pathogenesis of Chronic Obstructive Pulmonary Disease

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Running title: Neutrophils and COPD

Key words: proteinases, emphysema, chronic bronchitis, chemotaxis, phagocytosis

Word count: 3215

2 Figures

1 table

Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a common preventable disease characterized by an inflammatory infiltrate of the airways and progressive airflow obstruction. Whilst many inflammatory cells are implicated in COPD, the neutrophil is by far the most abundant and has been extensively associated with disease pathogenesis. Neutrophil products are thought to be key mediators of inflammatory changes in the airways of COPD patients, causing pathological changes such as emphysema and hypersecretion of mucus. Furthermore, both bacterial colonization and acute exacerbations of COPD are associated with increased neutrophil numbers and markers of activation, further highlighting their role in disease.

High rates of bacterial colonization and infective causes of acute exacerbations are observed in some patients with COPD despite this abundance of anti-bacterial neutrophils, raising the suggestion that neutrophil functions may be impaired in COPD. Exploring this hypothesis is complicated by the wide variety of functions exhibited by the neutrophil; impairment of any one of these could result in impaired bacterial clearance.

There is a need for new therapeutic strategies in COPD. Further explorations into neutrophil function in both health and COPD is allowing us to understand its role in disease pathogenesis and to elucidate whether this key inflammatory mediator represents a viable therapeutic target to prevent disease progression.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) remains a significant global health challenge. It has not seen the same improvements in morbidity and mortality as many other chronic inflammatory diseases and only one novel drug class has reached market in the past twenty years(1). After 25 years of smoking approximately 30 – 40% of adults will have developed COPD(2) but despite most patients having this shared risk factor, COPD is heterogeneous in presentation, age of onset and speed of decline. Whilst the disease is defined by the presence of airflow obstruction, patients can be divided into recognized clinical phenotypes. These include those with a predominance of emphysema(3), obstructive bronchiolitis (3, 4), the presence or absence of chronic bronchitis, evidence of bacterial colonisation of the lower airways with potentially pathogenic bacteria, those who experience frequent exacerbations(5) and those who experience a faster decline in lung function(6). Clinical phenotypes are stable within individual patients(6) and cluster within families(7) and thus are likely to reflect genetic traits. Indeed, genome wide association studies in COPD have identified a large number of signals for genes associated with lung development, lung parenchyma formation and repair and epigenetic regulation such as the inositol phosphate pathway(8, 9). These studies suggest there may be a wide range of therapeutic targets in different subsets of COPD patients, providing hope for personalised medicine. However drug development is costly and there are concerns about the affordability of targeting many different molecules for small groups of patients, especially in low income countries where the burden of COPD is rising fastest(10). This raises the question as to whether there might be a more common targetable mechanism across disease phenotypes which could provide a treatment for larger number of patients.

Inflammation in COPD

Most pro-inflammatory mediators and immune cells have been shown to be raised in lung secretions taken from patients with COPD(11) and this inflammation is heightened and self-sustaining in smokers who are susceptible to COPD in contrast to those smokers who are not (12, 13). However, while many cells and mediators have been implicated in COPD pathogenesis at some level, few have reliably demonstrated their importance as therapeutic targets in human studies. For example, despite the promising resistance to COPD-like lung damage shown by TNFα receptor (14) or IL-1 receptor 1 knock-out mice (15) studies targeting these individual mediators in unselected cohorts of patients with COPD have been disappointing with TNFα and IL-1 receptor 1 inhibition showed no improvements in disease endpoints(16, 17). This might suggest that, unlike murine models, only sub groups of patients will respond to individual mediator-based therapies. In support of this concept, there is variability in inflammatory patterns between patients, even when matched for age, smoking status and disease severity(18) which might reflect specific genetic traits as shown in some studies of TNFα (19) and IL-1β (20) polymorphisms.

To complicate matters further, there is significant intra-patient variability in the concentration of plasma and sputum mediators and cells on a day to day basis. Some mediators increase while others decrease suggesting the variability not only reflects dilution but also fluctuations in the specific components of the inflammatory load (18, 21). This supports an alternative explanation to the negative trial results reported to date, where there is so much redundancy, compensation and overlap within the complex inflammatory storm that is established COPD(22) that end-cell effects

can be driven by an alternative cytokine, should one be abrogated. For example, toll-like receptors, TNFα and IL-1 signalling to NF-κB all converge on a common IκB kinase complex that phosphorylates the NF-κB inhibitory protein IκBα, despite the upstream signalling components being to a large part receptor-specific(23). The effects of these mediators are synergistic and inhibiting one has not proved efficacious enough to impact robustly on cellular inflammation or COPD disease progression. Potentially targeting the functions of the end-cell and not the intermediatory cytokine might be more effective and there is a strong rationale for targeting the neutrophil in COPD.

Classical neutrophil functions in health

Neutrophils are the most abundant leukocyte, accounting for 70% of all circulating white blood cells. They are short-lived cells (with a half-life of around eight hours) with basal production of 1-2 x 10^{11} neutrophils/day in health; though this can increase to 10^{12} during infection(24-26). Following myeloblastic differentiation in the bone marrow, the mobilisation of terminally differentiated neutrophils into the circulation is tightly controlled by bone marrow signals and circulating growth factors including those between neutrophil CXCR4 and bone marrow stromal cell CXCL12 causing cell retention and neutrophil CXCR2 resulting in neutrophil release(27, 28). Neutrophils are characterised by the presence of a multi-lobed nucleus and granular cytoplasm, due to the presence of Azurophillic (primary), Specific (secondary) and Gelatinase (tertiary) granules, as well as secretory vesicles. These granules and vesicles contain a complex and specialised arsenal of proteins which facilitate neutrophil migration from the systemic circulation through the dense extracellular matrix to areas of inflammation, and then permit microbial killing and tissue remodelling and degradation, as listed in Table 1. This is not an "all or nothing response" and neutrophils require different levels of activation (and subsequent calcium mobilisation) to release granules, with secretory vesicles requiring the least stimulation, mobilising to facilitate migration and adhesion, while azurophil granules (which are the most cytotoxic) requiring the most stimulation.

Table 1. The contents of human neutrophil granules and secretory vesicles

Constituents	Azurophil	Specific	Gelatinase	Secretory
Matrix proteins	Elastase Proteinase 3 Cathepsin G Cathepsin D Defensins Lysozyme Myeloperoxidase Bacterial permeability increasing protein (BPI) Azuricidin α1-antitrypsin β-glucoronidase Phospholipase A2	Collagenase Gelatinase hCAP-18 Histaminase Heprainase Lactoferrin Lyoszyme NGAL β2 microglobulin	Gelatinase Lysosyme β2 microglobulin	Plasma proteins
Membrane proteins	CD63 CD68 Presenilin 1 V-type H+-ATPase	CD11b/CD18 Cytochrome b_{558} fMLP-R G-protein$_{\alpha}$-subunit Leukolysin VAMP-2	CD11b/CD18 Cytochrome b_{558} fMLP-R VAMP-2 V-type H+-ATPase	Alkaline phosphatase CD11b/CD18 CD16 CD10 CD13 CD14 fMLP-R

				C1q-R Cytochrome b_{558}

Legend. The contents of the different sub-types of neutrophil granule, divided into membranous and matrix (cytosolic) proteins. R, receptor; hCAP-18, human cathelicidin protein; VAMP-2, vesicle-associated membrane protein 2. fMLP, N-formylmethionyl-leucyl-phenylalnine

Neutrophils are guided towards inflammation in a gradient-dependent manner by chemotactic signals (30). Circulating neutrophils cross the endothelium at sites of inflammation via the Leukocyte Adhesion Cascade (LAC), whereby neutrophils interact with endothelial cells at sites of high inflammatory signalling, facilitating passage across the endothelium and stimulating signalling cascades within the neutrophil associated with antimicrobial armament and activation. It is unclear whether the LAC occurs in the pulmonary circulation however, due to the small diameter of the capillaries found there, which may influence neutrophil function (31).

Neutrophil homeostasis is also regulated by signals from tissue resident macrophages. When neutrophils become activated they release IL23 which stimulates IL-17 production. IL-17 induces release of G-CSF, causing further mobilisation of neutrophils into the blood stream to assist with clearing an infection (19,20).

Changing perceptions of the neutrophil

Neutrophils in COPD

Neutrophils are considered central to the pathogenesis of COPD. Airway neutrophilia is a feature of COPD regardless of the clinical phenotype, severity of disease, rapidity of decline or age of onset. Their numbers and products in sputum and airway lavage fluid correlate with disease severity, evident in the degree of airway obstruction, decline in FEV_1 and severity of emphysema present (32-35). Within the airway wall, confirmation of a neutrophilic presence has been more enigmatic, with some studies confirming(36) and others refuting their presence (37). However, neutrophils are not tissue resident, and inconsistencies could reflect the short life span of these cells. To address this, studies have focused on tissue collection at times of significant neutrophil recruitment to the airways in COPD (during acute exacerbations), and here numbers have been shown to be consistently raised (38). Increased levels of human neutrophil lipocalin (HNL) and myeloperoxidase (MPO), products of neutrophils degranulation, in the sputum of COPD patients suggest increased neutrophil activity in COPD airways (39). Concordantly, 18-fluorodeoxyglucose positron emission tomography studies of neutrophilic inflammation in COPD show enhanced uptake in the emphysematous regions of the lungs and correlate with measures of disease severity (40). Elegant pathological studies by Hogg et al suggest small airways dysfunction and destruction may precede the development of emphysema and airflow obstruction, and may reflect the earliest pathological changes in the lungs of patients with COPD (41). Neutrophils have also been implicated here, showing a relationship with neutrophilic infiltration and tomographic measures of air trapping (42).

Neutrophils and co-morbidities of COPD

Potential targets and therapeutic challenges – CXCR2 data. From trial

Activated neutrophils degranulate during migration towards inflammation and infection, during frustrated phagocytosis and NETosis, releasing serine proteases and elastolytic enzymes (e.g. neutrophil elastase [NE], matrix metalloproteases [MMPs] 8 and 9, proteinase-3) into the airways of COPD patients (43, 44). Excessive activity of these enzymes in COPD airways is thought to degrade elastin and type III collagen, leading to destruction of alveolar tissue and consequently centrilobular emphysema, apparent in many cases of COPD (43, 44). Serine proteases are also potent stimulants of mucus secretion in the airways, and cigarette smoke is thought to have direct effects on cilia, shortening them and reducing mucociliary clearance (45-47). The esulting mucus accumulation further obstructs the airways and increasing the risk of bacterial colonisation, contributing to the inflammatory environment (45, 48). Figure 1 summarizes the inflammatory interactions thought to underlie COPD.

Alveolar macrophages also contribute to and may orchestrate this inflammatory milieu by the release of inflammatory mediators and macrophage-specific and scavenged proteinases, recruiting more neutrophils to the airways, which in turn promote monocyte recruitment, thus leading to a vicious cycle of inflammatory damage.

COPD is also associated with a number of chronic inflammatory co-morbidities, including cardiovascular disease, type 2 diabetes, osteoporosis and periodontitis; greater prevalence of these co-morbidities are seen in COPD patients even once contributory factors (such as age, smoking and sedentary lifestyle) have been taken into account. It has been hypothesized that the pulmonary inflammation present in COPD may “overspill” systemically, leading to the development of different co-morbidities (with the type most likely dependent on individual susceptibility and exposures). Interestingly, many of the co-morbidities shared with COPD are also characterized by neutrophilic inflammation. For example, neutrophil products are raised acutely following a myocardial infarction and predict outcomes(49), neutrophil receptor expression and function are altered in diabetes(50), neutrophil lymphocyte ratios are inversely related to bone density in elderly people(51) and neutrophilic inflammation appears central to the development of periodontitis(52).

As demonstrated in figure 1, macrophages(53), T cells(54), B cells and auto-immunity(54, 55), and eosinophils(56) are all implicated in COPD. The degree to which a certain cell is involved in individual patients may reflect varying genetic predispositions or environmental exposures which drive disease within that individual. However, just as with each clinical phenotype, and with the majority of co-morbidities associated with COPD, no matter what cell type has been implicated neutrophils remain at the heart of the disease. In the landmark paper by Brightling discussing eosinophilic signals in COPD(56), average airway cells constituted of 2.4% eosinophils, 21.5% macrophages, 0.4% T-lymphocytes and 67.9% neutrophils.

The association between COPD and neutrophils is further evidenced by Alpha-1 Antitrypsin Deficiency (AATD). Alpha-1 Anti-trypsin is an anti-protease that directly antagonizes the proteolytic activities of NE on a one to one molar basis, and is thought to provide greater than 90% of the protective mechanisms against NE in the lungs (44, 57, 58). AATD is the most extensively documented genetic risk factor for COPD, where the inhibition of NE proteolysis predisposes to increased NE-mediated degradation of extracellular matrix and development of emphysema (58). Emphysema occurs even in the absence of smoking in some individuals and rates of decline in lung function are faster in those who smoke. Furthermore, there is evidence that emphysema progression is slowed by augmentation with replacement Alpha-1 Anti-trypsin(59) although the effect of augmentation on FEV_1 decline is less clear(60).

Of note, the persistently progressive and self-perpetuating inflammatory pattern observed in ex-smoking patients who develop COPD is not observed in ex-smokers who do not develop COPD(61, 62), suggesting a differential inflammatory response in individuals who are susceptible to disease in comparison to those who are not. Furthermore, only 25-50% of smokers develop COPD(63) and COPD also occurs in non-smokers(64), hence chronic exposure to cigarette smoke is neither sufficient alone to cause disease nor necessary for disease development, and other factors must play a role in disease development. In keeping with this, familial and genome association studies support the presence of a number of inherited traits that either promote or protect the host from COPD (65). Our current understanding of these traits is limited because genetic studies have not identified polymorphisms that are common in a large proportion of COPD patients. Furthermore, there is evidence that lung development (with early childhood and potentially in utero stimuli) contributes to disease burden (66). This has led to the orphan disease hypothesis of COPD(67); many potential genetic drivers and developmental factors, combined with the appropriate environment trigger, lead to the clinical symptoms and airflow obstruction that defines disease. Such a diverse array of potential susceptibility factors is challenging, both in terms of understanding disease pathogenesis and developing treatment strategies, and there is great interest in finding a unifying therapeutic target for the highest number of patients. The neutrophil may represent such a potential candidate target for therapeutic modulation.

Bacterial Colonisation and Acute Exacerbations

Bacterial colonisation of the lower respiratory tract is common in COPD; approximately 20-30% of stable COPD patients have a positive bacterial sputum culture and 60% show evidence of lower respiratory tract bacteria using culture independent assays. Bacterial colonisation correlates with more severe airflow obstruction, a more pronounced decline in lung function, worse health status and increased incidence of acute exacerbations (68-73). Bacterial colonisation is also associated with worsened pathological changes: increases in inflammatory cytokines are associated with neutrophil recruitment (such as IL-8 and LTB_4) and activation (TNFα), increased mucus secretion(74), reduced ciliary beat frequency(75), and epithelial damage(76, 77).

Many sufferers of COPD experience acute periods of exacerbation of respiratory symptoms, thought to be partly caused by an increase in the inflammatory infiltrate of the airways. For the majority of exacerbations (as high as 78%), this is induced by bacterial or viral infection, although many patients suffer exacerbations in the absence of identified infection (78-80). Acute exacerbations of COPD are significant medical events, often resulting in hospitalisation and occasionally mortality (81).

Irrespective of aetiology, airway neutrophilia shows a clear increase during exacerbations of COPD (38, 82). There is also evidence of increased matrix metalloproteinase-9 (MMP-9) activity, the neutrophil proteolytic enzyme (44). Furthermore, increased airway neutrophilia appears to be associated with critical expiratory flow limitation, dynamic lung hyperinflation, and consequently increased respiratory distress (68, 78, 83).

Neutrophil Functions in Health and COPD

Not all smokers develop COPD even when matched for exposure and there are differences in neutrophilic inflammatory burden in ex-smokers with and without COPD, therefore differential neutrophil functions are implicated in COPD pathogenesis.

Once within inflamed tissue, the neutrophil utilizes chemotactic gradients created by host-derived inflammatory cytokines (e.g. IL-8) and bacterial products (e.g. Lipopolysaccharide [LPS], N-formyl-methionyl-leucyl-phenylalanine [fMLP]) released from the site of infection, to migrate towards invading pathogens (30). These chemoattractant molecules bind their cognate receptors inducing functional changes in the neutrophil (30) such as the assembly of oxidative burst machinery (84). External gradients are amplified within the cell, allowing accurate mobilisation of internal structures (pseudopods for migration, granules for phagolysis) towards the inflammatory or infectious insult and facilitating full use of functions such as phagocytosis, production of toxic oxidative products, release of bactericidal granular proteins and production of neutrophil extracellular traps (NETs) (30).

The neutrophil granule system is designed to store highly toxic substances within the cell, facilitating fast release in response to invading pathogens (29). Granules are traditionally divided into four sub-types: azurophilic (primary)(29, 85) and specific (secondary)(29, 86, 87) granules contain predominantly bactericidal products (e.g. myeloperoxidase, α-defensins, lactoferrin), whereas gelatinase (tertiary)(30, 88) granules and secretory vesicles(89-91) contain products that aid neutrophil migration and endothelial transmigration (e.g. metalloproteases [MMPs], β_2-integrins, FcγIII [CD16]). Different granule sub-types are mobilized and released dependent on cytosolic free calcium levels, which allows coordination of granule release to match cellular requirement, for example mobilisation of azurophilic granules occurs only once the neutrophil comes into contact with a pathogen (92). Details of the contents of neutrophil granules are provided in table 1 (29).

Reactive Oxygen Species (ROS) are released both into phagosomes and externally during migration, degranulation and NETosis (see later). NADPH oxidase acts as a channel for electrons from the cytosol into phagosomal vacuoles, stimulating reduction of oxygen (O_2) to the superoxide anion O_2^- (93). Superoxide can then dismutate, a process accelerated by the enzyme superoxide dismutase (SOD), to form the highly oxidative hydrogen peroxide (H_2O_2), which can react further (the MPO-halide-hydrogen peroxide antibacterial system) to form strongly bactericidal hypohalous acids (e.g. HOCl) (85, 94-98). The role of ROS and the NADPH oxidase in elimination of pathogens is long-believed to be a dominant killing mechanism in neutrophils, however the exact role of these processes in neutrophil bacterial killing has come under recent scrutiny with some research supporting their role as facilitatory rather than obligatory (96).

Phagocytosis is an active, receptor-dependent process through which a phagocyte internalizes material into membrane-bound vacuoles (99). Direct interactions between neutrophilic pattern recognition receptors (PRRs) and surface-expressed pathogen-associated molecular patterns

(PAMPs) can generate signalling cascades that lead to ingestion of the target pathogen, a process termed 'unopsonized' phagocytosis (30, 100, 101). Phagocytosis of opsonized particles is a much faster process; ingestion of an IgG-opsonized particles by a neutrophil can take less than 20 seconds (102). The most extensively studied opsonin receptors of neutrophils, the Fc receptors (e.g. FcγRIIA [CD32], FcγIIIb [CD16]) bind IgG-bound particles triggering a signalling cascade involving the tyrosine kinase Syk and phosphatidylinositol 3-kinase (PI3K). Complement molecules also opsonize pathogens to phagocytic killing by interacting with neutrophil complement receptors (e.g. CR1 [CD35], CR3 [CD11b/CD18]) (103-106). Binding activates a signalling cascade involving phospholipase D (PLD), diacylglycerol (DAG) and the GTPase Rho. Both IgG and complement mediated phagocytosis result in cytoskeletal rearrangements that facilitate particle ingestion (107, 108).

Phagosome maturation refers to the equipping of the nascent phagosome with the antimicrobial potential required to eliminate a pathogen (103). Unlike the unquestionable acidification observed in the macrophage phagosome(103, 109), the neutrophil phagosome undergoes a transient alkalisation, despite evidence of V-ATPase proton-pumping activity, and antimicrobial activity is thought to be predominantly acquired through delivery of neutrophil granules and the NADPH oxidase machinery (30, 110, 111). Membrane trafficking supports the controlled delivery of bactericidal granular proteins into the phagosome (92, 112). These processes are depicted in Figure 2.

Neutrophils are also able to produce extracellular traps (NETs), consisting of a backbone of uncondensed chromatin to which bactericidal products such as cathepsins, MPO and nuclear histones are bound (113). Microscopic studies suggest NETs are responsible for the killing of a wide range of pathogens, including Gram-negative(113) and Gram-positive(113, 114) bacteria as well as fungi(115), however the process of NETosis occurs later in neutrophil activation than other killing processes such as phagocytosis or generation of ROS (116). NETs may also have detrimental effects on the host by exposing host molecules (i.e. DNA) within regions of active inflammation, introducing risk of autoimmunity. NETs have been implicated in cases of systemic lupus erythematosus (SLE), sepsis-induced hepatotoxicity and deep vein thrombosis (117-120).

There is a paradox that airway secretions from patients with COPD are neutrophil replete, correlating closely with disease severity, but that airway colonisation and infections are common (43, 121-123). Hence, it is likely that neutrophils present in COPD airways are impaired, weakening antimicrobial function and contributing to lung damage, and there is evidence supporting this hypothesis. COPD neutrophils demonstrate migratory inaccuracy, able to migrate towards chemotactic signals with greater speed, but decreased accuracy and velocity than neutrophils from age-matched healthy controls (124, 125). *In vitro* modelling suggests this results in a longer and more convoluted migratory path, increased secretion of damaging digestive enzymes and a delay in bacterial killing processes, potentially responsible for the increased rates of bacterial colonisation and infective exacerbations observed in COPD patients (124). Flow cytometric studies demonstrate enhanced respiratory burst in neutrophils of COPD sufferers as compared to healthy smokers(126), and furthermore increased markers of oxidative activity both in COPD airways and systemically suggest increased ROS-producing capacity in stable disease (127). Evidence in acute exacerbations is less clear, with some studies(128) suggesting the 'frequent exacerbator' phenotype of COPD demonstrates decreased oxidative burst in comparison to both healthy controls and stable COPD patients, and others demonstrating increased oxidative burst in active exacerbations (129, 130).

Similarly, there is conflicting evidence of NETosis; increased quantities of NETs and NET-producing neutrophils are observed in the sputum of both stable and exacerbating COPD patients(131, 132), however when isolated from blood, neutrophils of exacerbating patients demonstrate attenuated NET-producing ability (133). A potential explanation for this is an impaired clearance of NETs by DNAses(119) rather than increased production, but this is currently unexplored. The efferocytosis function of alveolar macrophages however, which mediates clearance of neutrophils(134), is thought to be impaired in COPD (135). The resulting accumulation of dead neutrophils in the airways may lead to secondary necrosis and excessive release of neutrophil granules and pro-inflammatory cytokines, contributing to an increasing neutrophilic infiltration and promoting an inflammatory environment (135, 136). There is limited data about the phagocytic functions of COPD neutrophils. Ingestion of opsonized *E. coli* and *Candida* species have been found to be reduced in COPD neutrophils compared to age-matched healthy controls (137, 138). However, neither Venge et al. nor Muns et al. observed any difference between the phagocytic abilities of COPD neutrophils and controls(139, 140), suggesting any defect may be both stimulant and assay dependent.

If neutrophil functions are dysregulated in COPD, favouring inflammation but impeding bacterial clearance, understanding the reasons for this is crucial to allow treatment. Genome wide studies have not consistently shown polymorphisms in pathways implicated in neutrophil functions, suggesting there are no common neutrophil-based genetic disorders causing COPD. However, transcriptional changes in neutrophil genotype have recently been described that appear to effect function in lupus patients(141), and it may be that the combination of multiple genetic factors, in themselves insufficient to cause disease, lead to epigenetic changes in neutrophils following environmental exposures which lead to long-term alterations in gene expression, perpetuating the inflammatory damage seen in COPD. Understanding these changes may unlock new strategies for COPD treatment.

Conclusion

Neutrophils are innate immune cells that have been widely implicated in the pathogenesis of COPD. They are a feature of all disease phenotypes and neutrophilic inflammation is described in many of the shared co-morbidities. Their ability to damage lung parenchyma as part of the inflammatory pathology of COPD has been well-described and there is mounting evidence of neutrophil dysfunction in COPD, which might explain the continued inflammatory response. All these data suggest the neutrophil may represent a unifying therapeutic target in COPD, but the necessary functions of these cells make this target a challenging one. Normalizing their activity whilst maintaining their ability to participate in host defence may be a crucial step in preventing COPD progression.

Declarations

AB, GMW have nothing to declare. ES has received funding from the Medical Research Council, British Lung Foundation , Wellcome Trust and has received non-commercial funding from GSK and Pfizer.

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Figure Legends

Figure 1 Inflammatory Airways of COPD. Inhaled irritants such as cigarette smoke induce inflammation in the airways, the degree of which is affected by lung development and epigenetic factors (A). Cigarette smoke stimulates release of cytokines from bronchial epithelial cells (B), including TGF-β which induces excessive collagen production from fibroblasts and consequently fibrosis of the small airways (C). Bronchial epithelial cells are also thought to recruit eosinophils to the airways via release of IL-33, although the exact role of the eosinophil in COPD is unclear (D). Alveolar macrophages are activated both by direct interactions with cigarette smoke and through cytokine signalling from bronchial epithelial cells (E). As well as further

recruitment of circulating monocytes (F), alveolar macrophages work with bronchial epithelial cells to recruit neutrophils in vast quantities (G). Neutrophils, aided by alveolar macrophages, secrete proteolytic and elastolytic enzymes (H) that mediate destruction of alveolar parenchyma (I) and induce hypersecretion of mucus within the airways (J), both contributing to airway obstruction. It has also been hypothesized that this pulmonary inflammation 'overspills' systemically (K), resulting in associated inflammatory co-morbidities, for example diabetes, myocardial infarction (MI) and reduction in bone density.

Figure 2 Phagosome Maturation. The neutrophil NADPH oxidase machinery, activated by delivery of its membrane-bound components to the phagosome, pumps electrons into the phagosomal space to generate toxic ROS. Membrane trafficking allows delivery of primary and secondary granules to the phagosomal membrane, which release a variety of microbicidal proteins into the phagosomal space. MPO released from primary granules reacts with ROS to further produce highly toxic substances . Adapted from(112). All abbreviations given in text if appropriate.